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Silicification of wood adopted for barrel production using pure silicon alkoxides in gas phase to avoid microbial colonisation

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A B S T R A C T

The paper presents a new approach, covering wood with silica-based material in order to protect it from spoilage due to microbial colonisation and avoiding the loss of the natural features of the wood. Wood specimens derived from wine barrels were treated with methyltriethoxysilane in gas phase, leading to the deposition of a silica nanofilm on the surface. ^{29}Si and ^{13}C solid state Nuclear Magnetic Resonance and Scanning Electron Microscope–Energy Dispersive X-ray analysis observations showed the formation of a silica polymeric film on the wood samples, directly bonding with the wood constituents. Inductively Coupled Plasma-Mass Spectroscopy quantification of Si showed a direct correlation between the treatment time and silica deposition on the surface of the wood. The silica-coated wood counteracted colonisation by the main wine spoilage microorganisms, without altering the migration from wood to wine of 21 simple phenols measured using a HPLC-Electrochemical Coulometric Detection.

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1. Introduction

Silica-based sol–gel materials are particularly useful for surface modification of traditional materials, as shown by certain examples in the fields of electronics, light interaction and protection from corrosion (Mahltig et al., 2008; Gurav et al., 2010; Sanchez et al. 2011). In the case of wood, the main methods involve dipping the materials into a gelling solution of silica precursors. Severe conditions are generally required to favour the hydrolysis of precursors and the condensation of siliceous unties with the formation of the silica layer. Panov and Nasko (2006) describe the silicification of wood with a mixture of ethyltriethoxysilane and diethyldietoxysialne, obtaining a valuable reduction in the water adsorption of wood and protecting it from fungal attack. Similar results were obtained, using other siloxanes, by Donath et al. (2010), Tshabalala et al. (2003), and Saka and Ueno (1997). In all works, a high temperature, over 80 °C, combined with high pressure (up to 12 bar) were required to allow deep impregnation of the wood by sylane, ensuring stable silicification. Therefore, these treatments are not suitable for certain applications involving wood, especially when the nature, or the use, of the objects makes it necessary to maintain the surface properties.

A valuable alternative is the direct utilisation of pure silicon alkoxides in gas phase. This solution has been found to be particularly useful, thanks to the possibility of operating in biocompatible conditions to coat viable cells, and requires the reliability of surface OH or embedded H_2O (Carturan et al., 2004; Avnir et al., 2006). This approach has the advantage of completely covering the surface of the material, independently of its weight and geometrical form (Callone et al., 2008). Moreover, the original extreme reactivity of silicon alkoxides is preserved and immediate formation of the wood–oxygen-bond can therefore be surmised. This aspect is not secondary, as the occurrence of real chemical bonds between the deposited silica layer and the wood guarantee the mechanical stability of the coating in the event of chemical, mechanical and thermal stress. An important aspect of wood protection using surface treatments regards the avoidance of biological degradation. In this case the sol–gel derived layer can provide a matrix for entrapment of biocide components, so a combination of features are obtained: the silica coating provides mechanical, thermal and fire protection and simultaneously the biocide components integrate the quality of the protection.

In this work we report on a new way of exploiting the general method for silica coating using gaseous silicon alkoxides (Carturan et al., 2006) to the treatment of wood surfaces. The paper deals with the deposition methods of organic modified silica, evaluation of the molecular structure of the inorganic matrix, possible chemical interaction with wood components using solid state NMR, study of

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the bioactivity of the samples as protection against deposition and colonisation by different microorganisms recognised as spoilage agents in the food industry.

Our attention focused on oak wood involved in the production of barrels used to make wine. *Barriques*, small wine barrels with a nominal volume of 225 L, are still produced using the traditional procedure with staves of *Quercus*, following prolonged seasoning of the wood. To produce *barriques* the staves must be flexible, so the wood is subjected to heat treatment, carried out by direct exposure to flame during the assembly of barrels. This procedure gives the *barriques* special proprieties and encourages direct effects on wine quality (Singleton, 1995). It is known that the permanence of wine inside barrels leads to a complex combination of chemical reactions, which directly involve the components of wood or, more generally, are indirectly mediated by wood, thanks to the particular environment that it helps to create inside *barriques*. Despite these precious effects on wine quality, the use of barrels leads to some problems. The porosity and inertness of wood favour the growth of a complex microbiota. New barrels are normally free from spoilage microorganisms but after few months of wine storage, growth of unwanted yeast or bacteria up to 10^3–10^4 cells for cm^2 can already be measured (Renouf et al., 2006; Oelofse et al., 2008) and the sanitization techniques currently available have generally been shown not to be very effective (Guzzon et al., 2011).

Wood silicification using pure silicon alkoxides in gas phase may represent a feasible solution. Previous results have demonstrated that the silica film obtained was uniform, with adequate mechanical and chemical stability (Callone et al., 2008). The texture resulting from the condensation of methyltriethoxysilane (MTES) unities provides narrow porosity and excludes the migration of microorganisms thought the silica film, while at the same time fully preserving chemical exchanges (Carturan et al., 2004; Callone et al., 2008). A detailed description of this new approach to wood silicification is proposed here, accompanied by chemical/physical characterisation of the material obtained. Considering that the work is still at an experimental stage, conducted in the laboratory using wood samples, all the experiments are discussed by comparing the behaviour of wood samples coated with silica gel with untreated wood samples, in order to highlight the properties caused by the presence of the silica nanofilm bonded to the wood surface.

2. Materials and methods

2.1. Materials

Methyltriethoxysilane ($CH_3Si(OEt)_3$), ethanol, and other chemicals were purchased from Sigma–Aldrich as reagent-grade products and used without further purification. All reagents and apparatus involved in the microbiological tests were sterilised at 121 °C × 15 min before use. Yeasts and acetic acid bacteria were cultured in Modified Wallenstein Broth Medium (Oxoid); lactic bacteria cells were cultured in MRS Broth (Oxoid).

2.2. Wood coating

Two types of wood samples were used in this study, according to the goal of each experiment. The first type of sample was made up of oak wood blocks (50 × 40 × 30 mm) obtained by cutting up medium toasted barrique staves (G & P Garbellotto S.p.A); these samples were used in all the experiments. The specimens were washed in water (60 °C × 15 min) and dried (30 °C × 24 h). Five sides of each sample were covered with paraffin wax (low mp 70–80 °C, Sigma–Aldrich); only one side (surface 20 cm^2) remained available for silica coating. The wood samples were hermetically sealed in the reaction chamber (Fig. 1). A second type of samples was used only for the tests on the release of phenols in wine. This samples was made up of medium toasted oak staves (dimensions 5 × 5 × 50 mm; Tonnellerie National Italia). MTES vapours were obtained by treating a MTES solution in the heating chamber at 90 °C. The vapours were transferred using a N_2 gas flux (0.5 L/min) in the reaction chamber containing the wood samples. The treatment was performed for 4 different durations (from 5 to 20 min); the temperature in the reaction chambers remained below 40 °C for the entire treatment duration. Finally, the wood samples were dried for 60 min at 25 °C.

2.3. Physical and chemical characterisation

Microscopic observation of samples was carried out using a SMZ80 stereoscope, equipped with a DSFi1 Digital Camera (Nikon). Physical characterisation of the treated wood block was performed as proposed by Donath et al. (2010), the weight of samples was determined using a Mettler Toledo PM460 balance. The modifications in the weight and volume of the samples after exposure to silica alkoxide gas were described as a weight percent gain (WPG) and bulking effect (B) (Donath et al., 2010) when,

$$\mathrm{WPG} = \frac{m_{\mathrm{treated}} - m_{\mathrm{untreated}}}{m_{\mathrm{untreated}}} \times 100\%$$

$$B = \frac{V_{\mathrm{treated}} - V_{\mathrm{untreated}}}{V_{\mathrm{untreated}}} \times 100\%$$

Micro-structural analysis was carried out using a JEOL JSM 5500 Scanning Electron Microscope (Oxford Instruments Analytical) at various magnifications at 10 kV, 20 kV for Energy Dispersive X-ray analysis (EDX).

^{29}Si and ^{13}C solid state NMR spectra were recorded with a Bruker 400 WB spectrometer with carrier frequency of 400.13 MHz (1H) equipped with a double resonance probe. Before analysis, the specimens were dried (120 °C × 24 h) and ground using a M20 Universal Mill (IKA). Samples were packed in 4 mm zirconia rotors and spun at 6 kHz. ^{29}Si CPMAS spectra were obtained with pulse length 4.3 μs, scan delay 10 s; 6 k scans with contact time of 5 ms. SP sequence was used for quantitative analysis. Q_8M_8 was used as an external secondary reference. Si units were labelled according to the usual NMR notation: Qn, Tn, with capital letters referring to the number of Si–O– bonds and *n* being the number of oxo-bridges. ^{13}C CPMAS spectra were acquired with 90° pulse length of 3.36 μs, contact time of 2 ms, 2000 scans and scan delay of 5 s. Adamantane was used as a secondary external reference.

Determination of silicon was carried out using Agilent 7500ce Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent Technologies). Before analysis, the wood samples were dried (120 °C × 24 h) and ground using a M20 Universal mill (IKA). Acid digestion of wood powder was performed in PTFE vessels using ultrapure nitric acid (69.5%, Merck) and hydrogen peroxide (30%, Merck). A rhenium solution (800 μg/L) was added as internal standard. Mineralization was performed in a high-throughput microwave reaction system (MARSXpress™, CEM) using the following thermal cycle: 12 min at 100 °C, 15 min at 150 °C, and 22 min at 210 °C.

2.4. Microorganisms and biological tests

The microorganisms belonged to the German Collection of Microorganisms and Cell Cultures (DSMZ), the Industrial Yeast Collection (DBVPG), and the ARS Culture collection (NRRL). Fermentation tests were performed at 25 °C in Yeast Medium broth

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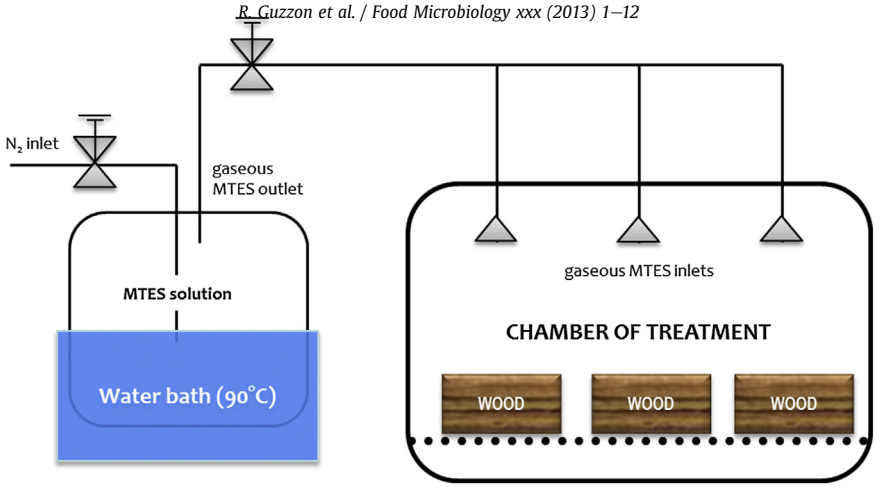

Fig. 1. Diagram of the apparatus involved in wood silicification by gaseous pure alkoxides.

(OXOID, composition: Yeast Extract 3 g/L, Malt Extract 3 g/L, Peptone 5 g/L, pH 6.2, glucose from 180 to 250 g/L). The amount of synthetic medium was adjusted to obtain a volume/surface ratio similar to that occurring in standard wine barrels; the wood blocks were immersed in Yeast Medium broth (YM) for one day before yeast inoculums (*Saccharomyces cerevisiae* ATCC 9763, inoculum concentration 2.5×10^6 CFU/mL) and maintained in this conditions for 7 days. The evolution of alcoholic fermentation was followed by measuring the mass loss related to CO_2 production (Bely et al. 1990). Fermentation curves were fitted using the modified Gompertz function (Zwietering et al., 1991) and the program Table Curve 2D™ (Systat Software) results were treated according to the Lineweaver–Burk method.

Tests of wood block contamination were performed by immersing the specimens in 250 mL of a culture of each microorganism listed in Table 5 (approx. 10^6 CFU/mL). The wood blocks were left in contact with the microbial suspension for 14 days at 20 °C. After incubation, microbiological analysis of the surface of the specimens was performed as stated in ISO 18593:2004. Microbiological determination was carried out by swab sampling of a defined surface (5 cm^2) or by Contact Petri Plate, according to the expected microbial contamination. In the case of swab sampling, the surface was delimited by a sterile paper lattice and completely sampled by rubbing the swab, previously moistened with sterile saline solution. The swab was immediately immersed in a vial containing 10 mL of peptoned water (1 g/L of mycological peptone, OXOID) and stirred for 5′ using an IKA (S) vortex at the maximum speed. The cellular suspension obtained was analysed through a plate count (OIV, 2012). Contact Petri Plates (Sarsted, D) containing Modified Wallenstein Broth Medium (OXOID) were placed on the wood samples and kept in contact with the surface of specimens using gentle pressure for 10 min. Then the Contact Petri Plates were incubated at 25 °C for 3 days or more, in accordance with the requirements of each species of microorganisms (OIV, 2012). Results were expressed as colony forming units per cm^2, using the lattice placed on the bottom of Contact Petri Plate as a reference. The analysis was repeated after gentle washing (3×100 mL of water at 20 °C) and thorough washing (3×1 L of water at 95 °C) of specimens with distilled sterile water.

Table 1
Chromatographic and electrochemical parameters of the simple phenols quantified by HPLC-ECD in wines after contact with wood specimens.

Compound	CAS N.	Retention time (min)	DL (ug/L)	Dominant channel potential (mV)
Gallic acid	149-91-7	3.5	20	100
Hydroxytyrosol	10597-60-1	5.4	10	200
Gentisic acid	490-79-9	6.7	2	100
Tyrosol	501-94-0	7.6	5	500
Catechin	154-23-4	9.6	5	500
Homovanillic acid	306-08-1	10.1	1	400
Vanillic acid	121-34-6	10.5	1	500
Caffeic acid	331-39-5	10.9	10	100
Syringic acid	530-57-4	12.2	20	400
Epicatechin	490-46-0	12.6	5	500
Vanillin	121-33-5	14.4	10	500
p-Coumaric acid	501-98-4	15.0	5	500
Syringaldehyde	134-96-3	15.9	5	400
Ferulic acid	501-98-4	18.0	20	400
Isopropiosiringone	23133-83-7	18.7	10	400
Tryptofol	526-55-6	21.4	1	500
Ellagic acid	476-66-4	21.7	100	300
Coniferaldehyde	458-36-6	23.5	3	400
Synapaldehyde	4206-58-0	24.7	1	300
4-Vinylguaiacol	7786-61-0	28.8	3	300
4-Allylsyringol	6635-22-9	31.1	5	300

2.5. Wood coating and the release of phenols in wine

In order to evaluate whether the proposed silicon coating of the inner cask surface represented a physical–chemical barrier capable of altering normal transfer phenomena between wood and wine during ageing two experiments were carried out. In the first experiment, wood samples coated with MTES as described above, were stored for 2 weeks in closed plastic bags, filled by 50 mL of white wine (*Chardonnay*, ethanol 12.5%, pH 3.48) in a 150 mL glass vial. The amount of wine was decided in order to approximate the usual surface/volume ratio of barrels. The head space inside the container was filled with nitrogen gas before capping. The mixture was shaken daily and the extraction process took 16 days at 15 °C before removal of the wood material. Wines were filtered 0.45 μm through a PTFE membrane. The same process was also performed with 10 untreated staves and the wine samples obtained in this way were used as a reference (control) for the extraction process. In the second experiment the contact time between wood samples (coated with MTES) and wine was prolonged for 3 months at 20 °C. Sampling was performed after 1, 2, and 3 months. The test was replicated 10 times.

In both cases, after contact with the wood samples, wines were analysed by adapting the HPLC method proposed by Larcher et al. (2007). The use of an HPLC (Alliance 2695 system; Waters Corporation, Milford) with a Coulometric Array Electrochemical detector (5600A; ESA, Bedford) equipped with eight porous graphite

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Table 2
Main features of wood samples obtained following different exposure times to silicon alkoxide vapours (Mean data ± SD, $n = 5$).

Sample	MTES gas exposure (min)	WPG[a] (%)	B[b] (%)	Si	
				(mg/cm^2)[c]	(Atom. weight %)[d]
Untreated wood	0	2.58 ± 1.13[a]	2.24 ± 0.52[b]	0.06 ± 0.01	–
Treated wood	5	1.79 ± 0.78[a]	2.76 ± 1.13[b]	0.79 ± 0.26	4.83 ± 1.83
	10	1.25 ± 1.63[a]	1.68 ± 0.58[b]	1.78 ± 0.47	5.68 ± 0.59
	15	2.52 ± 1.01[a]	2.06 ± 0.89[b]	2.25 ± 0.41	6.31 ± 2.26
	20	3.83 ± 1.89[a]	2.21 ± 1.47[b]	nd.	nd.

[a] Weight Percent Gain (data having same apex were not significantly different, Anova test $p < 0.05$).
[b] Bulking effect (data having same apex were not significantly different, Anova test $p < 0.05$).
[c] Data determined using ICP-MS.
[d] Data determined using SEM–EDX. nd. Not determined.

Table 3
^{29}Si NMR chemical shifts and related assignments.

Unit type	δ (ppm)	Rel. amount (%) *vs.* Impregnation time			
		20 min	15 min	10 min	5 min
T^2	−53.6	43.6	44.3	46.7	50.7
T^3	−62.3	56.4	55.7	53.3	49.3
DOC[a] (%)		81.2	81.4	82.2	83.6

[a] DOC: degree of condensation.

working electrodes (set at potentials between 100 and 800 mV) and a 150 × 3 mm, 2.7 mm pentafluorophenylpropyl fused-core particle column (Supelco Ascentis Express®) made it possible to detect 21 phenolic analytes present in all the samples, over the detection limits, among the 57 investigated (3,4-Xylenol, 4-Allylsyringol, 4-Ethycathecol, 4-Ethylguaiacol, 4-Ethylphenol, 4-Methylcatechol, 4-Methylguaiacol, 4-Methylsyringol, 4-Vinylguaiacol, 4-Vinylphenol, Acetosyringone, Acetovanillone, Caffeic acid, Catechin, Coniferyl alcohol, Coniferaldehyde, Cyclotene, Ellagic acid, Epicatechin, Esculetin, Ethyl vanillate, Eugenol, Ferulic acid, Furaneol, Gallic acid, Gentisic acid, Guaiacol, Homofuraneol, Homovanillic acid, Homovanillic alcohol, Hydroxytyrosol, Isoacetosiringone, Isoacetovanillone, Isoeugenol, Isopropiosiringone, Isopropriovanillone, m-Cresol, Methyl vanillate, o-Cresol, p-Carboxyphenol acid, p-Coumaric acid, p-Cresol, Phenol, Protocatechualdehyde, Protocatechuic acid, Scopoletin, Sinapic acid, Syringic acid, Synapaldehyde, Syringaldehyde, Syringol, Tryptofol, Tyrosol, Vanilli acid, Vanillin, Vanillyl ethyl ether). Table 1 gives the list of standards, retention times, detection limits (DL; calculated as 3 standard deviations of the water/ethanol signal, 8:1 analysed 10 times), and the main discharge potentials (dominant channel potentials) of the 21 compounds quantified.

3. Results and discussion

3.1. Coating of wood with silica film and characterisation of specimens

A diagram of the apparatus used for silica gas deposition, described in the experimental section, is presented in Fig. 1. The

Table 4
Cellular density found on wood surface before incubation in a highly concentrated culture of different oenological microorganisms with potential spoilage capacity (Mean data ± SD, $n = 3$).

Microorganism	Microbial contamination before washing (10^2 UFC/cm^2)	Sample	After gentile washing (10^2 UFC/cm^2)	After thorough washing (10^2 UFC/cm^2)
Debaryomyces hansenii CBS 767	1200 ± 280	Untreated wood	1000 ± 370	45 ± 17
		Coated wood	50 ± 18	ng.
Metschnikowia pulcherrima CBS 5833	280 ± 45	Untreated wood	220 ± 80	9.3 ± 3.4
		Coated wood	30 ± 11	ng.
Schizosaccharomyces pombe CBS 356	220 ± 60	Untreated wood	210 ± 77	2.0 ± 0.7
		Coated wood	30 ± 11	ng.
Zigosaccharomyces bailii CBS 680	970 ± 110	Untreated wood	800 ± 290	6.7 ± 2.5
		Coated wood	50 ± 18	ng.
Dekkera bruxellensis DBVPG 6705	350 ± 140	Untreated wood	330 ± 120	1.7 ± 0.6
		Coated wood	20 ± 7	ng.
Dekkera bruxellensis NRRL Y-1411	380 ± 110	Untreated wood	370 ± 140	1.2 ± 0.4
		Coated wood	20 ± 7	ng.
Brettanomyces spp. *DBVPG 4051*	400 ± 120	Untreated wood	410 ± 150	4.0 ± 1.5
		Coated wood	30 ± 11	ng.
Hanseniaspora uvarum NRRL Y-1614	490 ± 75	Untreated wood	510 ± 190	8.0 ± 3.0
		Coated wood	40 ± 15	ng.
Pichia membranifaciens NRRL Y-2026	580 ± 85	Untreated wood	430 ± 160	4.3 ± 1.6
		Coated wood	20 ± 7	ng.
Pichia guilliermondii CBS 2030	610 ± 160	Untreated wood	550 ± 200	5.0 ± 1.9
		Coated wood	10 ± 4	ng.
Hansenula anomala CBS 5759	1300 ± 280	Untreated wood	1100 ± 400	5.2 ± 1.9
		Coated wood	10 ± 4	ng.
Acetobacter acetii DSMZ 3508	120 ± 40	Untreated wood	100 ± 40	1.2 ± 0.4
		Coated wood	8 ± 3	ng.
Gluconobacter oxydans subsp. suboxydans NRRL B-72	130 ± 25	Untreated wood	100 ± 35	1.1 ± 0.4
		Coated wood	10 ± 2	ng.
Oencoccus oeni DMSZ 20252	130 ± 40	Untreated wood	120 ± 50	0.7 ± 0.3
		Coated wood	50 ± 20	ng.
Saccharomyces cerevisiae ATCC 9763	890 ± 85	Untreated wood	560 ± 180	1,5 ± 0.6
		Coated wood	20 ± 7	ng.
Pediococcus pentosaceus NRRL B-14009	160 ± 25	Untreated wood	140 ± 32	1.0 ± 0.4
		Coated wood	40 ± 18	ng.
Lactobacillus plantarum NRRL B-4496	110 ± 10	Untreated wood	100 ± 32	1.2 ± 0.4
		Coated wood	60 ± 12	ng.

ng.: no growth observed on contract Petri plate after sampling of wood blocks.

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Table 5
Kinetic parameters of alcoholic fermentation with *Saccharomyces cerevisiae* ATCC 9763 in synthetic media in the presence of wood specimens treated with MTES vapour.

Sample	V_{max} ($\times 10^{-5}$ M CO_2 s^{-1})	K_m (M^{-1})	Fermentation yield[a] (%)	Cell density in the media at 50% of AF ($\times 10^7$ UFC/mL)
No wood	1.36 ± 0.01	0.31 ± 0.01	64.2 ± 0.8	1.9 ± 0.1
Untreated wood	1.15 ± 0.01	0.30 ± 0.01	64.1 ± 0.9	1.9 ± 0.1
Coated wood (5 min)	1.16 ± 0.02	0.20 ± 0.02	63.1 ± 0.5	1.8 ± 0.2
Coated wood (10 min)	1.09 ± 0.01	0.19 ± 0.01	62.9 ± 0.9	1.8 ± 0.1
Coated wood (15 min)	1.20 ± 0.01	0.23 ± 0.01	63.2 ± 0.6	1.8 ± 0.1

[a] Calculated as ratio between Ethanol production (% vol/vol) and initial sugars content (°Brix).

samples were treated using the same experimental conditions for 5, 10, 15 and 20 min; then they were stored at room temperature and humidity for 30 days, protected from contamination by dust. The weight percent gain (WPG) and bulking effect (B) (Table 2) describe the evolution of the main physical properties of wood samples in relation to exposure to MTES gas flux. No variations in the weight and dimension of samples were observed, WPG and B index did not appear to depend on the coating treatments and were also found in samples exposed only to nitrogen gas flux (uncoated samples). They could thus be explained by taking into account the dehydration undergone by samples during treatment in the gaseous phase, as already observed by Callone et al. (2008) in the case of silica coating of calcium alginate micro beads. Anova test, performed considering 5 different populations of data according to the time of treatments of wood specimens, not found significant variations both in terms of weight and dimensions of wood specimens. The physical variations observed in the specimens were negligible, especially when compared with data on traditional treatment of wood with alkoxysilanes reported by other authors (Donath et al. 2010), which were higher by almost one order of magnitude. For our purposes, this result is particularly relevant, because one of the specific features of wine barrels is the fact that they are made by directly joining oak staves without the use of adhesives: in these conditions significant variations in the size of staves may causes fractures in the barrels and loss of tightness. After 1 month ageing of wood samples, elemental analysis showed the presence of silica on the wood surface (Table 2) to be almost absent in untreated wood samples. The amount of silica deposited increased over time, demonstrating that the coating process is time-dependent, with an interval of between 0 and 15 min. In order to evaluate the structure of the film deposited, SEM–EDX analysis was run. This is considered to be a valuable technique for studying wood modifications after the deposition of organ-silicon compounds (De Vetter et al., 2006); in this case SEM micrographics showed the effective silica deposition on the specimens. Fig. 2 gives some significant micrographs of our samples. At low magnification, silica-coated wood specimens were similar to the original untreated wood; by increasing the magnification it was possible to discriminate between the samples on the basis of the exposure time of the alkoxide in gas phase. The non-homogeneous profile of the untreated wood surface (Untreated wood, Fig. 2) was clearly coated with a siliceous layer. The sample treated for 20 min did not show the original porosity, fractures and waviness. Its surface appeared to be completely coated with the deposited silica layer. Moreover, some spherical particles were observed. EDX analysis indicated that these particles were made of silica. This may be attributed to long exposure of the sample to the reactive flux of silicon alkoxides: the amount exceeding the interaction with the wood surface was not influenced by geometrical features and was free of self-organisation in low free energy geometries, justifying the occurrence of the small spherical silica microspheres observed. Initially the continuous flux of reactive alkoxide caused coverage of the wood surface active groups, but subsequently the silane was deposited layer after layer, increasing the thickness of the inorganic layer and encouraging self-aggregation. In the context of our study, this may cause complete closure of wood porosity, preventing the wine–wood contact giving typical flavours to wine. This treatment was therefore considered to be outside the scope of our research and not considered for further analysis. The evolution of wood morphology documented by SEM observations is in agreement with previous works dealing with wood treated with silica derivatives to increase its resistance to biological attacks (Mai and Militz, 2004; Vignali et al., 2011). It is worth noting that the reduction in wood roughness and macro porosity were obtained

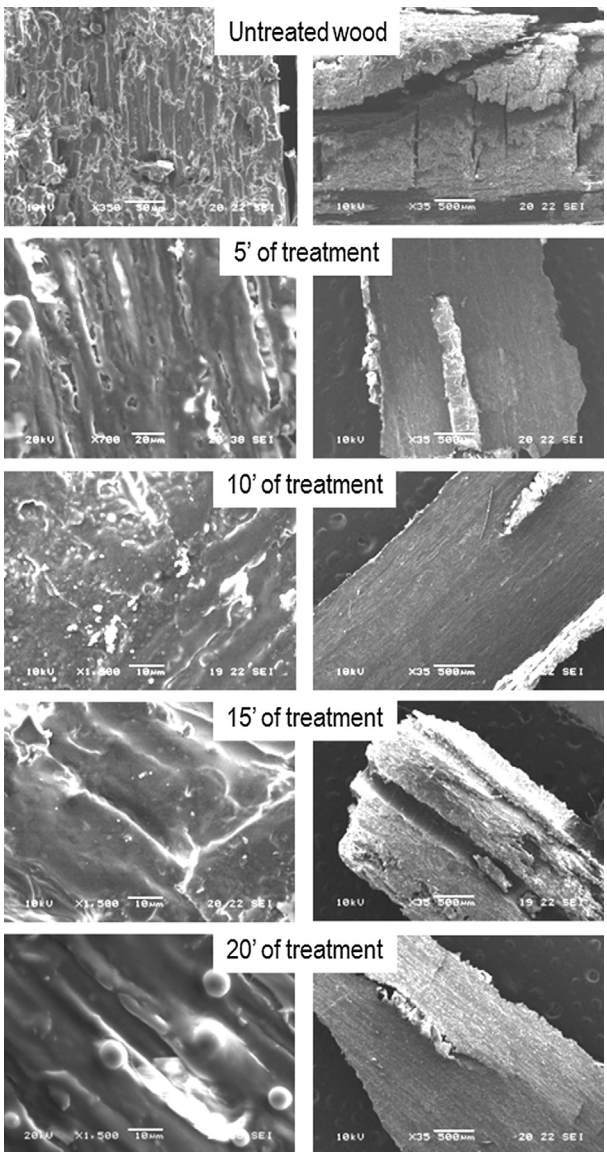

Fig. 2. SEM observations of wood samples after different exposure times to gaseous MTES.

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here in mild conditions and in a relatively short time, in comparison with previously reported data (Mohammed-Ziegler et al., 2003).

The chemical reactions involved in the coating of organic substrates (viable cells or structure made by organic polymers) by silicon alkoxide derivatives were already exhaustively described by some authors, with particular reference to the works Carturan (Carturan et al., 2004, 2006; Callone et al., 2008) and Avnir et al. (2006). Therefore a complete description of the chemistry of silica materials obtained by sol gel route likely to be repetitive and is out of the scope of this work. However, some consideration about the nature of the composite material obtained from the reaction between wood and MTES can be advanced by the help of data obtained by solid state NMR analysis and with particular attention to the potential applications in the field of food technology and winemaking.

i. The synthetic route of the composite material involves the hydrolysis of Si–OR groups of MTES and the condensation between Si–OH and –OR groups (Avnir et al., 2006). With the experimental conditions described here, the hydrolysis and the condensation of silica alkoxide was promoted by different factors: the high temperature of system (about 90 °C), by the gaseous state of silica precursors (MTES), and by the –OH groups and water molecules absorbed on the wood surface.
ii. ^{29}Si NMR spectra, recorded on the scratched surface grains of treated wood specimens (Fig. 3 and Table 3), indicates that the molecular structure of the silica portion of specimens was made up of T^3 [$H_3CSi(OSi)_3$] and T^2 [$H_3CSi(OSi)_2OR$] structural units; where R could be H, wood glucosidic units, or OEt. The latter option (R = OEt), can be excluded, since our samples were kept in the laboratory atmosphere for a very long time (more than 30 days) and the siliceous layer is very thin, so residual Si–OR groups were totally hydrolysed or condensed. The total absence of T^1 units strengthens the hypothesis that the material is chemically stable and free from residual reactivity.
iii. As shown in Fig. 4, the amount of silica deposited increased almost linearly with the exposure time to alkoxide gas flux. The linearity discovered can be used in further experiments to adjust the amount of silica matrix desired on the specific surface. According to Table 3, the ratio between T^3/T^2 units increased slightly with the treatment time. The increase in the thickness of the silica layer created a large number of condensed units as compared to the T^2 units, which are probably those exposed at the surface of the layer. This also means that the methyls bonded to silicon are too small to interfere (steric hindrance) with the formation of a homogeneous thick glassy silica layer.
iv. The ^{13}C NMR spectrum was also recorded (Fig. 5). In addition to the contributions relating to the principal components of the wood, at −5 ppm it clearly present the peak of Si–CH_3 bond, demonstrating the effective occurrence of the bond between the woody substrate and the siliceous polymer. At all events it confirmed complete hydrolysis of the Si–OEt groups, because the related OCH_2CH_3 carbon peaks are absent.

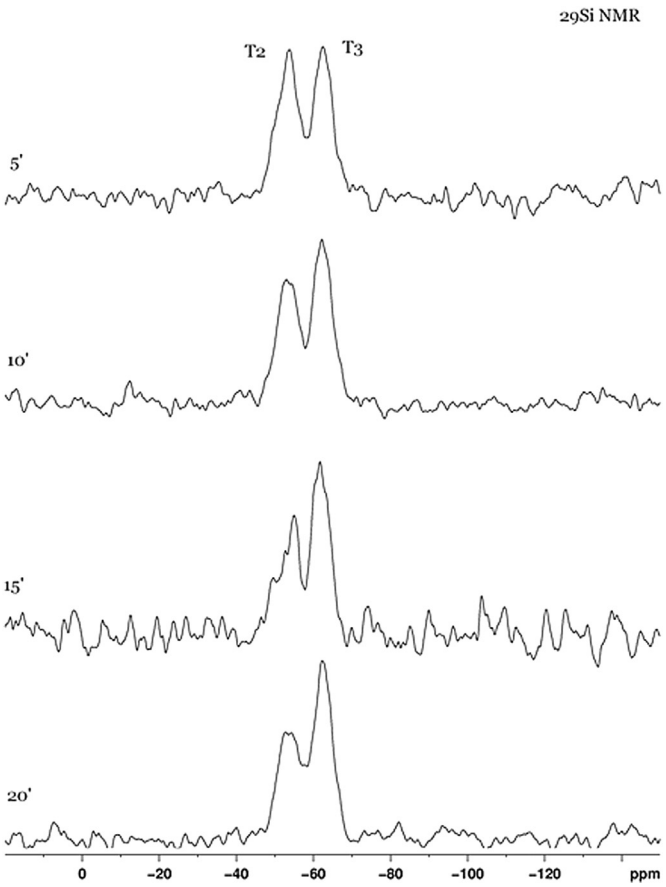

Fig. 3. ^{29}Si CPMAS NMR spectrum of wood samples after different exposure times to gaseous MTES.

In conclusion, the analysis of NMR data means that experimental parameters such as type of silica alkoxide, time of reaction, temperature were able to produce a stable silica layer bonded with wood derived from barrels. This is clearly indicated by the high Degree of Condensation (DOC) shown by the samples. This represents the weighted ratio between the silica units, i.e. $(3T^3+2T^2)/3$. It increased over time and was above 80% in all cases (Table 3). From the applicative point of view this evidence suggest that we obtained a "new" material, different from the original wood, and not only a coverage of a substrate by a protective layer. This result is independent from the nature of wood artifacts (*barriques*, large barrels, or other apparatus) because only linked to the intrinsic characters of the two components involved in the reaction (wood and MTES). Despite this, further tests in real conditions of winery are necessary to establish the effects of some empirical stresses due to the productive process, not carefully reproducible in laboratory conditions.

3.2. *Evaluation of the protection offered by the silica layer against microbial colonisation of wood*

Silicification of wood to protect it against biodegradation is already applied in some fields of industry (Mai and Militz, 2004; Mohammed-Ziegler et al., 2003). Here, the protection of wood offered by a silica layer was studied, focussing the attention on microorganisms recognised to be capable of spoiling wines (Oelofse et al., 2008; Guzzon et al., 2011). Wood specimens, exposed for 15 min to gaseous MTES, were immersed in a synthetic medium containing a high concentration of microorganisms; subsequently the samples were incubated for a long time to encourage extensive contamination of the wood by yeast or bacteria which reached the 3 log units in the majority of samples (Table 4). The same procedure was applied to samples of untreated wood to verify the different behaviour of materials in the presence of an abundant microbial population in the surrounding environment. After contact between the wood and microorganism cultures, the samples were gently

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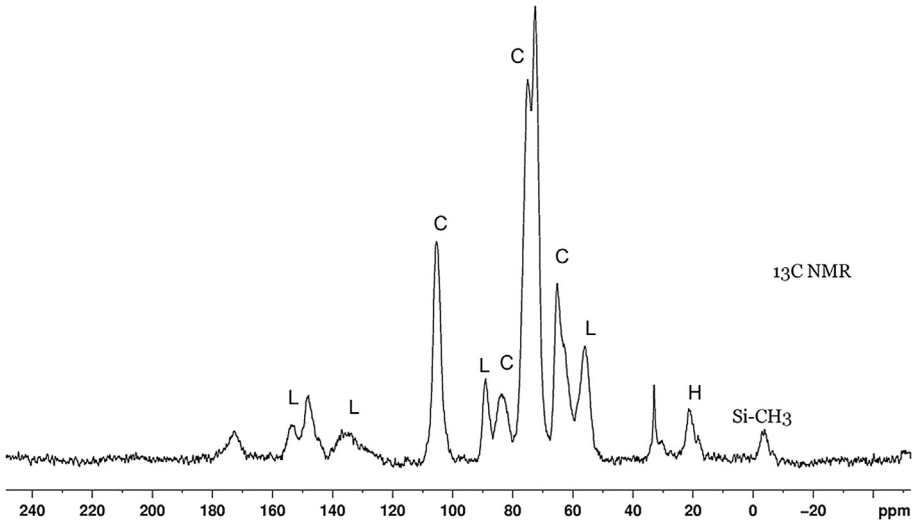

Fig. 4. ^{13}C CPMAS NMR spectrum of wood sample treated at 15 min, as a representative of all the samples (L = lignin, C = cellulose, H = hemicellulose).

washed to remove macroscopic residues and subjected to microscopic observations and microbiological assay. The images in Fig. 6 (stereoscopic observation of wood specimens, magnification 80×) show the differences in the adhesion of *Dekkera bruxellensis*, known as a wine spoilage yeast capable of producing biofilm (Joseph et al., 2007), occurring in silica-coated or uncoated wood specimens. Fig. 6A and C illustrate the surface of an uncoated wood specimen. The untreated wood surface (Fig. 6A) was covered with an extensive biofilm after incubation in a media containing *D. bruxellensis* cells (Fig. 6C). On the other hand, wood blocks coated with the silica film (Fig. 6B), and put in the same cellular suspension, did not display microbiological colonisation and biofilm formation (Fig. 6D). The microbiological analysis listed in Table 4 gives more detailed results. In both experiments (gentle or thorough washing) the untreated wood specimens showed a residual microbial population after cleaning treatments. This was expected, considering the physical features of the wood surface (rawness and porosity) and agree with previous experiments performed in a winery (Guzzon et al., 2011). Silica-coated specimens showed a reduction in microbial colonisation of almost 2 orders of magnitude already after gentile washing (Fig. 7), and a total disappearance of microbial contamination after thorough washing that, it should be noted, is performed only with hot water (65 °C), without uses sanitizing agents. Indeed, in the case of untreated samples the gentile washing is ineffective, eliminating about the 15% of microbial population. As expected, the thorough washing reduces the contamination of samples with a residual presence of microbes around the 2–5% of initial population (Fig. 7). However, this performance is not sufficient to allow a prolonged safety of wood because the value of cells density measured on wood specimens remained in the order of 2–3 log unities (Table 4). These results are even more significant if one considers that the microbial concentrations in the media surrounding wood specimens was at least 2–3 orders of magnitude higher than those considered dangerous in wine for the majority of spoilage microorganisms (Malfeito-Ferreira, 2011; Oelofse et al., 2008; Loureiro and Malfeito-Ferreira, 2003). The main hypothesis advanced to describe the protection against microbial colonisation given to wood by silica film involves

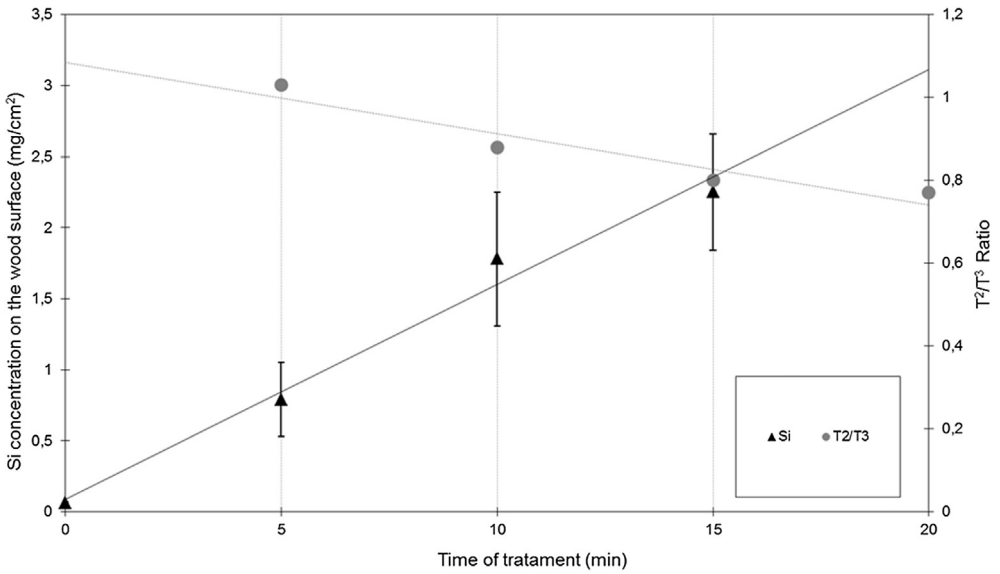

Fig. 5. Evolution of Si amounts and T^2/T^3 ratio on the basis of wood exposure time to silica vapours.

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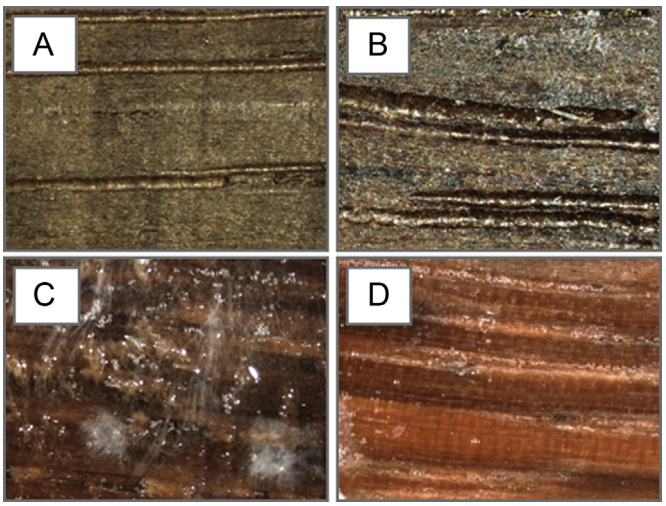

Fig. 6. Microphotography (Magnification 80X) of wood specimens before and after contamination by *Dekkera bruxellensis*. A. Untreated wood surface. B. Wood surface after coating with silica film. C. Untreated wood surface after 14 day contact with a 10^8 CFU/mL solution of *D. bruxellensis*. D. Wood surface coated with silica film after 14 day contact with a 10^8 CFU/mL solution of *D. bruxellensis*.

the specific features of silica layers produced using MTES. It is known that polymer obtained using MTES shown a hydrophobic nature, as demonstrated by Callone et al. (2008) in the study of migration of organic acid through MTES derived barriers. Here, the hydrophobicity of siliceous layers discourages the adhesion of cells to the surface of matrices, preventing the chemical interaction between cells and wood typical of the first steps in biofilm formation (Joseph et al., 2007); furthermore film deposition reduces wood macro porosity, excluding the deep penetration of the cells to the core of samples. Another important consequence of the hydrophobic nature of siliceous membrane is the reduction precipitation, on the surface of material, of inorganic salts of tartaric acid. This phenomena is common observed in oenology and causes the reduction of porosity of some apparatus (barrels, filters) with interferences with the chemical exchange. The data reported here agree with previous evidences obtained by the same authors (Guzzon et al., 2011) that demonstrated the durability of a bio reactor based on a composite material derived from silicon alkoxide in wine conditions.

3.3. Effects of silica coating on wood on the biological activity of S. cerevisiae

The siliceous membrane discourages the adhesion of the microorganisms trying to colonise the wood surface. However, in winemaking barrels are frequently used as containers during alcoholic fermentation of the wine. In this context, a specific test to check that the silica layers have not interfered with yeast activity during wine fermentation is opportune. In our experiments we performed a series of alcoholic fermentations in a model system deprived of any interferences to the activity of *S. cerevisiae* with the exception of wood samples. Synthetic media containing wood specimens (coated with a silica layer or untreated), varying the initial sugar concentration in the oenological range (Fig. 8A). The data of fermentation curves were modelled by the Gompertz equation, obtaining information about the V_{max} and the lag phase of each experiments. Data were also treated following the Lineweaver & Burk approach (Fig. 8B) to determine the main kinetic constants of the whole process (Table 5). No significant differences were found between the V_{max} of fermentation performed in the presence of coated or uncoated wood blocks. A few differences were found in relation to the test, i.e. alcoholic fermentation performed without wood, but the data did not appear to be relevant from the technological point of view. Likewise, the K_m and yield of fermentation (the conversion rate of sugars in alcohol) did not vary in the different trials and were in the typical range for *S. cerevisiae*. Measurement of cell density in the medium, after

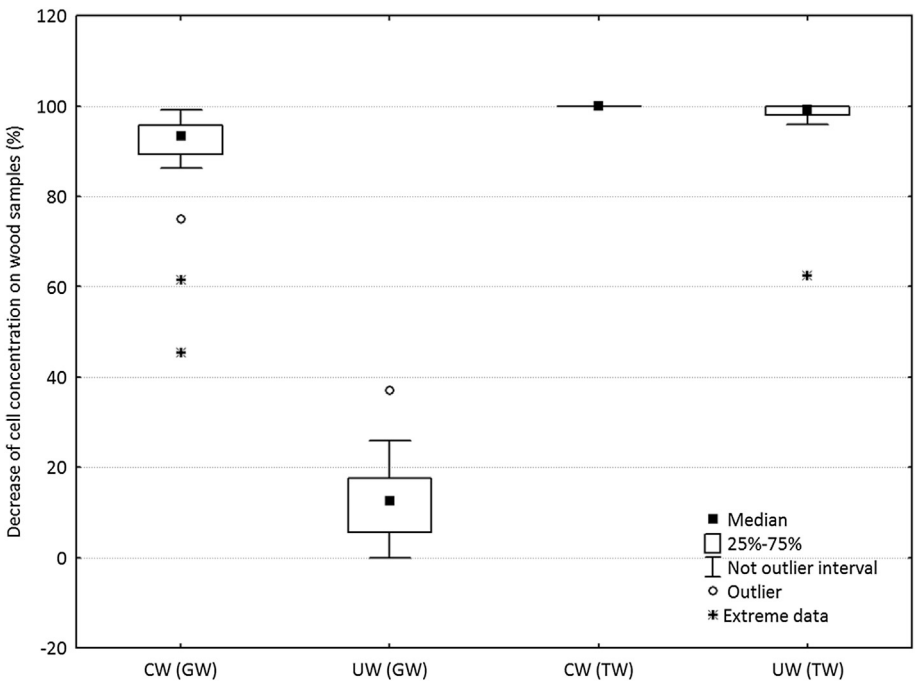

Fig. 7. Box plot of reduction of cell concentration observed on wood samples after washing. CW: coated wood samples, UW: untreated wood samples, (GW) gentile washing, (TW) thorough washing.

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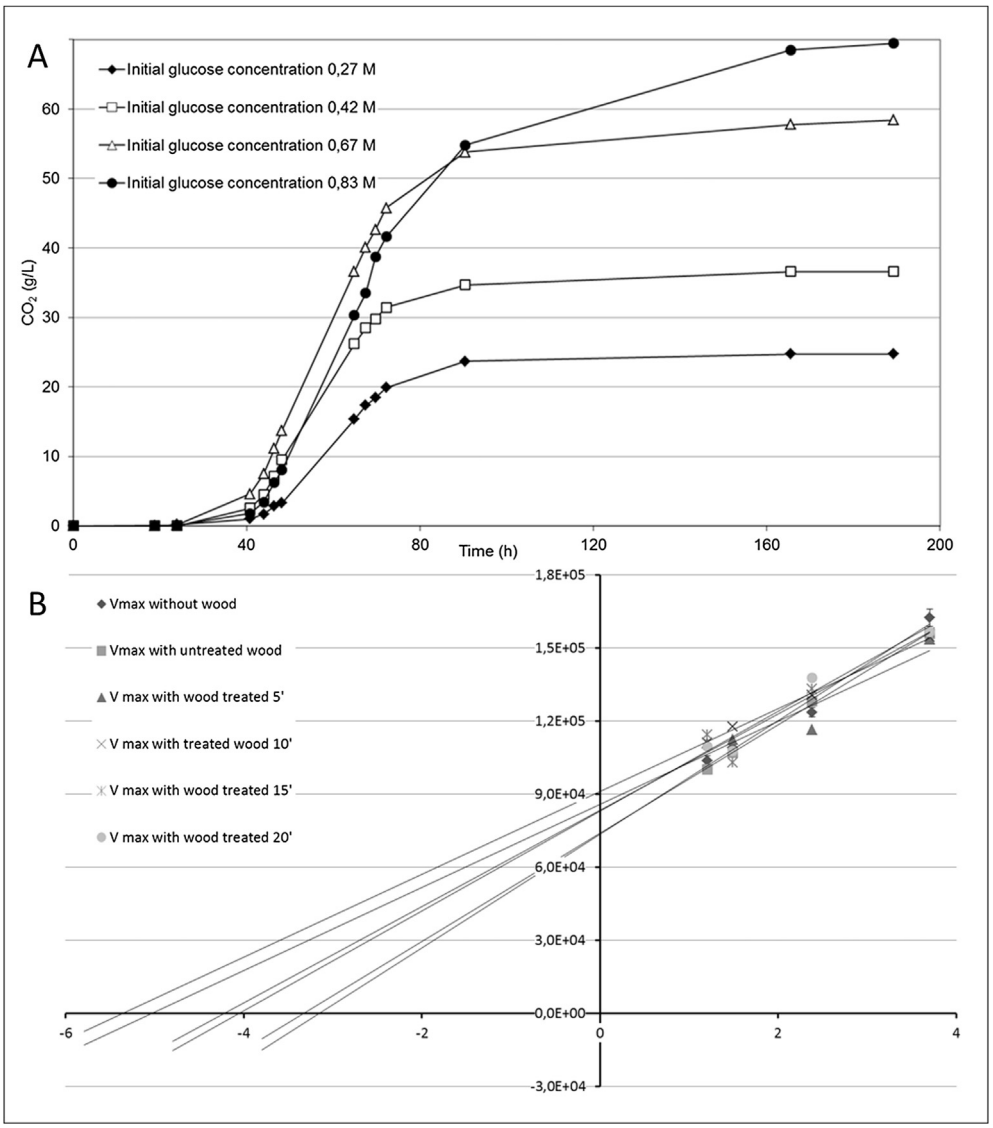

Fig. 8. A. Evolution of alcoholic fermentation performed in synthetic media with *Saccharomyces cerevisiae* in the presence of wood (15 min treatment). B. Lineweaver & Burk plot of alcoholic fermentation performed with *Saccharomyces cerevisiae* in synthetic media in the presence of wood samples.

consumption of 50% of sugars, dispelled doubts about the toxicity of silica derivate for cells present in the medium. The data showed a high concentration, generally higher than 10^7 CFU/mL, following the standard evolution of *S. cerevisiae* during alcoholic fermentation (Ribéreau-Gayon et al., 1998), indicating that the silica membrane did not have an adverse effect on active cells present in the medium.

3.4. Study of the interference of silica layers on the release of phenolic components in wine

Maintaining the chemical exchange between wood and wine is a key property of the siliceous membrane. Two sets of tests were therefore carried out in order to determine the modifications caused, in terms of the interaction between the wood and the surrounding media, due to the deposition of the siliceous membrane. Depending on the manufacturing process, which requires heat treatment of the wood using flame, the characteristics of barrels are extremely variable, which makes it difficult to obtain reproducible and representative results considering a limited number of wood specimens. In winemaking this problem is resolved by blending wines coming from different barrels, to reach the best equilibrium in the final product. In the present work, the laboratory conditions in which the tests were performed did not allow this approach. Therefore, tests were performed using were commercial oak staves chosen in order to guarantee a higher uniformity of behaviour in the experiments.

In the first test we focused our attention on the behaviour of siliceous layer bonded to the wood samples. About 20 simple phenols were recovered in quantifiable amounts in the samples of wines after a 16-day period of contact with wood specimens. No quantifiable amounts of eugenol were found in any wine samples; other molecules that potentially playing a role in the definition of wine aroma (*e.g.:* 2-furfurilthyol) were not analysed because made by yeast metabolism (Blanchard et al., 2001). Fig. 9 shows a comparison between the mean content of simple phenols with untreated and silica coated toasted oak staves. No statistical differences were found for the analysed compounds using the HSD Tukey test for unequal numbers, with the exception of homovanillic acid, which showed significant differences ($p < 0.01$) between the

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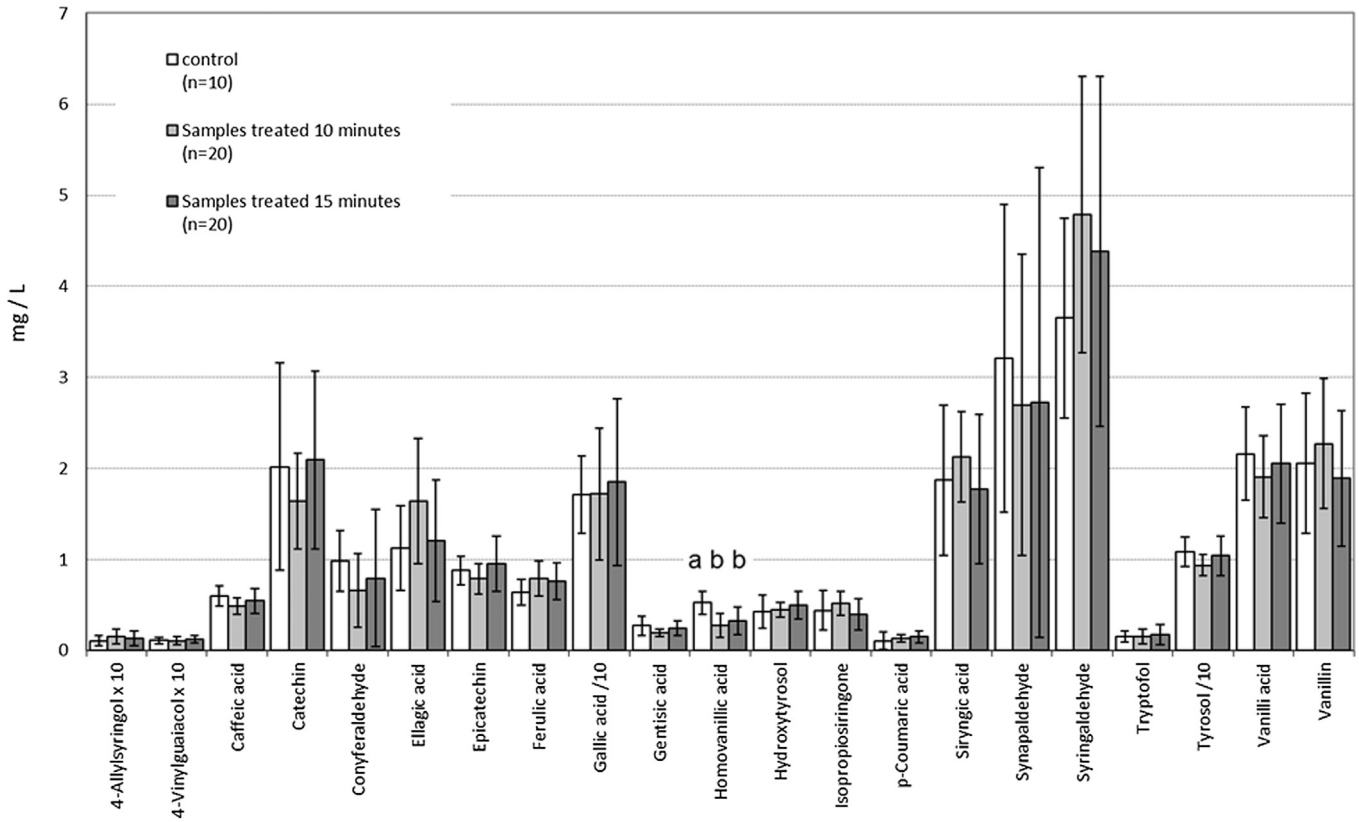

Fig. 9. Comparison between the mean phenol content (bars) in white wine after 16-days infusion with untreated (control; $N = 10$) and silica coated (treated; 10 and 15 min) toasted oak staves. Whiskers correspond to standard deviations. (For a better graphical representation the height of the bars of 4-allylsyringol and 4-vinylguaiacol are magnified 10 times, the height of the bars of gallic acid and tyrosol are reduced 10 times).

control and both the treated samples, without differences between the coating time at 10 and 15 min.

The goal of the second experiment was to describe the behaviour of the silica membrane over a prolonged contact time with wine (3 months). Analysis of the wines obtained showed considerable variability in the concentration of each individual compound in the 10 replicates of each trial, independently of the contact time. The 21 molecules recovered are representative of the phenolic acid released from wood to wine. In 9 cases we observed a direct correlation between the concentration in wine and the contact time between wood and wine. In the other cases the amount of compounds found in wine did not vary between the first and the 3rd sample, probably because the phenols reached a balance in terms of the distribution between wood and wine (Fig. 10). Statistical analysis of the data obtained (subjected to Tukey's HSD test) showed a general absence of differences caused by the silica coating on the phenolic profile of wines. Only in 4 cases did we identify a significant difference between the two samples (samples treated for 15 min and untreated samples) but these cases appeared to be randomly distributed in the data set and consequently devoid of any technological significance. It is however possible to conclude that the siliceous membrane did not interfere with the chemical exchange between wine and wood and that the intrinsic variability between barrels is higher than any possible modification caused by the presence of the silica membrane, even when the contact with the wine is prolonged for several months.

The ambivalent behaviour of the siliceous membrane, allowing the migration of small molecules but at the same time useful for counteracting the migration of structures of high biological significance, such as cells, antibodies or proteins, was expected and agreed with previous evidence (Carturan et al., 2004; Cellesi and Tirelli, 2006). Dire et al. (1997) had already demonstrated that siliceous gel materials prepared from MTES had a low surface area, narrow porosity and mechanical behaviour similar to silicon rubber. More recently, Callone et al. (2008) studying the fermentative activity of yeast (*S. cerevisiae*) or lactic acid bacteria (*Oenococcus oeni*), found that a MTES-derived siliceous membrane, obtained from gaseous deposition of silica precursor on alginate, allowed the free migration of glucose, malic acid and peptides, ensuring the complete maintenance of metabolic activity of entrapped cells. Another contribution helping to understand the potential of siliceous membrane was offered in the 2011 by Guzzon et al. (2012) demonstrating the dimensional inhibition of contact between lactic bacteria entrapped in silica/alginate micro beads and lysozyme, added to the fermentation medium. Considering the molecular structure of lysozyme, which has a molecule size of about 30 Kda, the authors confirmed the evidence proposed by Carturan et al. (2004) as regards the dimensions of siliceous membrane pores.

In this work, despite the fact that the silica film covered the wood specimens uniformly (Fig. 2), the texture resulting from condensation of $[(CH_3)Si]-(OEt)_n$ ($n = 1,2,3$) MTES units provided narrow porosity while fully preserving chemical exchanges, without interfering with the process of wine ageing, during which the phenolic fractions released by barrels play a fundamental role. With the current state of research, the hypothesis suggesting that some modifications in the phenolic profile of wood occur due its reaction with silica derivate cannot be excluded. However, we did not observe any significant impact on the phenolic profile of the wines produced (Fig. 9), especially in the case of molecules with the highest organoleptic activity, or over prolonged contact times (*e.g.*

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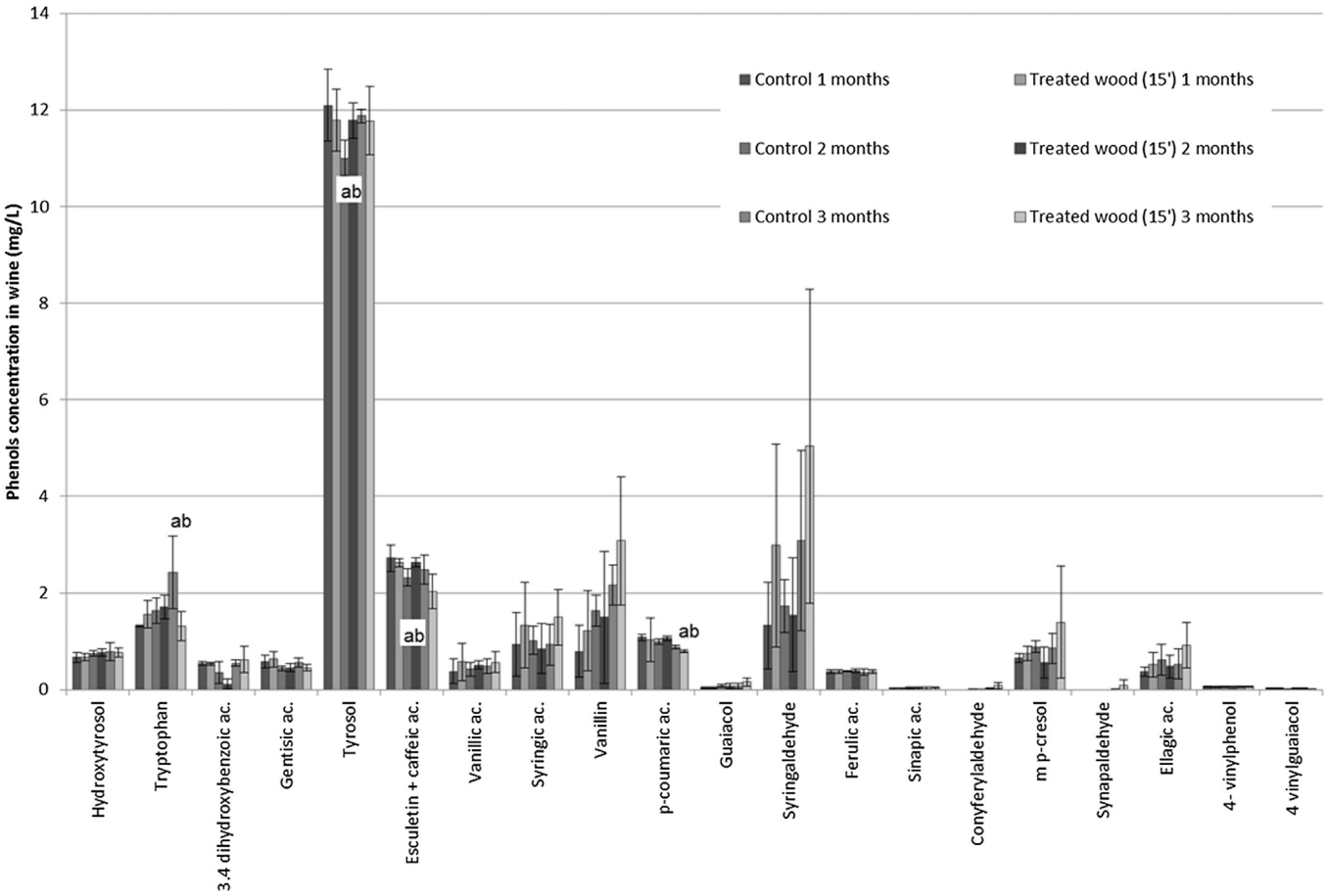

Fig. 10. Mean phenol content in wine after 3 monthly contact with wood blocks from barrels, treated with MTES ($n = 5$), in comparison with the phenolic profile of wine in contact with untreated wood samples (control, $n = 5$).

vanillin, Fig. 10C). Observing the same data, and particularly the significant variance characterising the data obtained from untreated wood, it is clear that the natural variability of wood covers any changes resulting from treatment with MTES coating, making them not technologically relevant.

4. Conclusions

In the food industry the attention of technicians and consumers is now directed at products obtained using low-impact procedures, guaranteeing the healthiness of food. In this context, traditional sanitization treatments, based on the extensive use of chemicals and/or energy, and applied to beverage and food production, no longer appear to have a future. New approaches to the prevention of microbial contamination are therefore required. In this work a feasible solution is suggested, thanks to pioneering experimentation aimed at synthesizing new composite materials obtained from the gaseous deposition of a silica membrane on wood used to produce barrels involved in the ageing of highly valuable beverages such as wine, beer or distillates. The physical/chemical characterisation of the materials produced made it possible to demonstrate that an effective silica matrix is formed inside the wood. The biological tests showed the effective action of silica membrane against biological contamination and at the same time, the absence of interference with the biological process occurring in the environment surrounding the coated wood. The tests demonstrated that the chemical exchanges between the wood and environment essential for making high quality products were maintained. The proposed composite material could be used successfully for different applications in various fields of food technology, where wood remains a useful and sometimes irreplaceable material. However, considering the particular sensitivity of consumers to innovations introduced in the food and oenological industry, an accurate scaling-up of this promising solution from the laboratory to the industrial scale will be required to make this solution available to technicians.

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